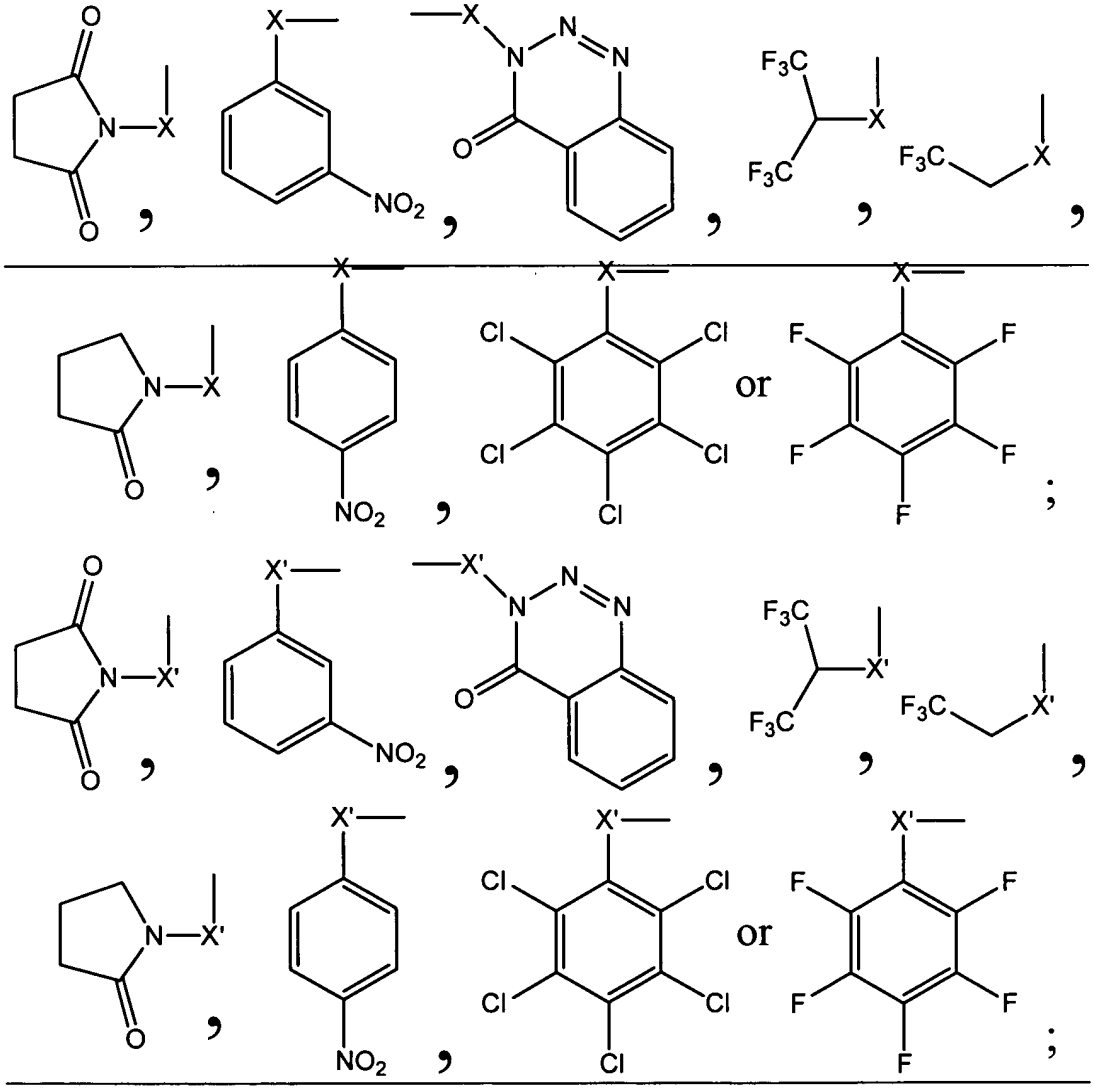
II. AMENDENT OF THE SPECIFICATION

Please amend the paragraph beginning on page 8, line 6 to page 9, line 2 as follows:

The reactive group of a labeling reagent can be an amine reactive group. For example the amine reactive group can be an active ester. Active esters are well known in peptide synthesis and refer to certain esters that are easily reacted with the N-α amine of an amino acid under conditions commonly used in peptide synthesis. The amine reactive active ester can be an N-hydroxysuccinimidyl ester, a N-hydroxysulfosuccinimidyl ester, a pentafluorophenyl ester, a 2-nitrophenyl ester, a 4-nitrophenyl ester, a 2,4-dinitrophenylester or a 2,4-dihalophenyl ester. For example, the alcohol or thiol group of an active ester can have the formula:



wherein X' is O or S, but preferably O. All of the foregoing being alcohol or thiol groups known to form active esters in the field of peptide chemistry wherein said alcohol or thiol group is displaced by the reaction of the N-α-amine of the amino acid with the carbonyl carbon of the ester. It should be apparent that the active ester (e.g. N-hydroxysuccinimidyl ester) of any suitable labelling/tagging reagent described herein could be prepared using well-known procedures (See: Greg T. Hermanson(1996). "The Chemistry of Reactive Groups" in "Bioconjugate Techniques" Chapter 2 pages 137-165, Academic Press, (New York); also see: Innovation And Perspectives In Solid Phase Synthesis, Editor: Roger Epton, SPCC (UK) Ltd, Birmingham, 1990). Methods for the formation of active esters of N-substituted piperazine acetic acids compounds that are representative examples of labelling reagents of the general formula: RP-X-LK-Y-RG, are described in co-pending and commonly owned United States Patent Application Serial No. 10/751,354, incorporated herein by reference.

Please amend the paragraph at page 10, lines 15 to 28 as follows:

The reporter either comprises a fixed charge or is capable of becoming ionized. Because the reporter either comprises a fixed charge or is capable of being ionized, the labeling reagent might be isolated or used to label the reactive analyte in a salt or zwitterionic form. Ionization of the reporter facilitates its determination in a mass spectrometer. Accordingly, the reporter can be determined as an ion, sometimes referred to as a signature ion. When ionized, the reporter can comprise one or more net positive or negative charges. Thus, the reporter can comprise one or more acidic groups or basic groups since such groups can be easily ionized in a mass spectrometer. For example, the reporter can comprise one or more basic nitrogen atoms (positive charge) or one or more ionizable acidic groups such as a carboxylic acid group, sulfonic acid group or phosphoric acid group (negative charge). Non-limiting examples of reporters comprising a basic nitrogen include, substituted or unsubstituted, morpholines, piperidines or piperazines.

Please amend the paragraph at page 14, line 29 to page 15, line 6 as follows:

These types of mass spectrometers may be used in conjunction with a variety of ionization sources, including, but not limited to, electrospray ionization (ESI) and matrix-

assisted laser desorption ionization (MALDI). Ionization sources can be used to generate charged species for the first mass analysis where the analytes do not already possess a fixed charge. Additional mass spectrometry instruments and fragmentation methods include post-source decay in MALDI-MS instruments and high-energy CID using MALDI-TOF(time of flight)-TOF MS. For a recent review of tandem mass spectrometers please see: R. Aebersold and D. Goodlett, *Mass Spectrometry in Proteomics. Chem. Rev. 101:* 269-295 (2001). ~~Also see United States Patent No. 6,319,476, herein incorporated by reference, for a discussion of TOF-TOF mass analysis techniques.~~

Please amend the paragraph at page 16, lines 13-31 as follows:

In some embodiments, analytes can be determined based upon daughter-ion fragmentation patterns that are analyzed by computer-assisted comparison with the spectra of known or "theoretical" analytes. For example, the daughter fragment ion spectrum of a peptide ion fragmented under conditions of low energy CID can be considered the sum of many discrete fragmentation events. The common nomenclature differentiates daughter fragment ions according to the amide bond that breaks and the peptide fragment that retains charge following bond fission. Charge-retention on the N-terminal side of the fissile amide bond results in the formation of a b-type ion. If the charge remains on the C-terminal side of the broken amide bond, then the fragment ion is referred to as a y-type ion. In addition to b- and y-type ions, the CID mass spectrum may contain other diagnostic fragment ions (daughter fragment ions). These include ions generated by neutral loss of ammonia (-17 amu) from glutamine, lysine and arginine or the loss of water (-18 amu) from hydroxyl-containing amino acids such as serine and threonine. Certain amino acids have been observed to fragment more readily under conditions of low-energy CID than others. This is particularly apparent for peptides containing proline or aspartic acid residues, and even more so at aspartyl-proline bonds (Mak, M. et al., *Rapid Commun. Mass Spectrom., 12:* 837-842) (1998). Accordingly, the peptide bond of a Z''-pro dimer or Z''-asp dimer, wherein Z'' is any natural amino acid, pro is proline and asp is aspartic acid, will tend to be more labile as compared with the peptide bond between all other amino acid dimer combinations.

Please amend the paragraph at page 18, lines 8-16 as follows:

When the analyte of interest is a protein or peptide, the relative lability of bonds X and Y can be adjusted with regard to an amide (peptide) bond. Bond X, bond Y or both bonds X and Y can be more, equal or less labile as compared with a typical amide (peptide) bond. For example, under conditions of dissociative energy, bond X and/or bond Y can be less prone to fragmentation as compared with the peptide bond of a Z''-pro dimer or Z''-asp dimer, wherein Z'' is any natural amino acid, pro is proline and asp is aspartic acid. In some embodiments, bonds X and Y will fragment with approximately the same level of dissociative energy as a typical amide bond. In some embodiments, bonds X and Y will fragment at a greater level of dissociative energy as compared with a typical amide bond.

Please amend the paragraph at page 18, lines 8-16 as follows:

For example, the proteolytic enzyme trypsin is a serine protease that cleaves peptide bonds between lysine or arginine and an unspecific amino acid to thereby produce peptides that comprise an amine terminus (N-terminus) and lysine or arginine carboxyl terminal amino acid (C-terminus). In this way the peptides from the cleavage of the protein are predictable and their presence and/or quantity, in a sample from a trypsin digest, can be indicative of the presence and/or quantity of the protein of their origin. Moreover, the free amine termini of a peptide can be a good nucleophile that facilitates its labeling. Other exemplary proteolytic enzymes include papain, pepsin, ArgC, LysC, V8 protease, AspN, pronase, chymotrypsin and carboxypeptid[e]ase C.

For example, a protein (e.g. protein Z''') might produce three peptides (e.g. peptides B, C and D) when digested with a protease such as trypsin. Accordingly, a sample that has been digested with a proteolytic enzyme, such as trypsin, and that when analyzed is confirmed to contain peptides B, C and D, can be said to have originally comprised the protein Z'''. The quantity of peptides B, C and D will also correlate with the quantity of protein Z''' in the sample that was digested. In this way, any determination of the identity and/or quantify of one or more of peptides B, C and D in a sample (or a fraction thereof), can be used to identify and/or quantify protein Z''' in the original sample (or a fraction thereof).

Please amend the paragraph at page 21, line 25 to page 22, line 6 as follows:

In some embodiments, the relative quantitation of differentially labeled identical analytes of a sample mixture is possible. Relative quantitation of differentially labeled identical analytes is possible by comparison of the relative amounts of reporter (e.g. area or height of the peak reported) that are determined in the second mass analysis for a selected, labeled analyte observed in a first mass analysis. Put differently, where each reporter can be correlated with information for a particular sample used to produce a sample mixture, the relative amount of that reporter, with respect to other reporters observed in the second mass analysis, is the relative amount of that analyte in the sample mixture. Where components combined to form the sample mixture ~~is~~ are known, the relative amount of the analyte in each sample used to prepare the sample mixture can be back calculated based upon the relative amounts of reporter observed for the ions of the labeled analyte selected from the first mass analysis. This process can be repeated for all of the different labeled analytes observed in the first mass analysis. In this way, the relative amount (often expressed in terms of concentration and/or quantity) of each reactive analyte, in each of the different samples used to produce the sample mixture, can be determined.

Please amend the paragraph at page 21, line 25 to page 22, line 6 as follows:

For example, the analyte might be a peptide that resulted from the degradation of a protein using an enzymatic digestion reaction to process the sample. Protein degradation can be accomplished by treatment of the sample with a proteolytic enzyme (e.g. trypsin, papain, pepsin, ArgC, LysC, V8 protease, AspN, pronase, chymotrypsin or carboxypeptid[e]ase C). By determination of the identity and amount of a peptide in a sample mixture and identifying the sample from which it originated, optionally coupled with the determination of other peptides from that sample ~~sample~~, the precursor protein to the degraded peptide can be identified and/or quantified with respect to the sample from which it originated. Because this method allows for the multiplex determination of a protein, or proteins, in more than one sample (i.e. from a sample mixture), it is a multiplex method.

Please amend the paragraph at page 27, lines 1-8 as follows:

In some embodiments, the whole process can be repeated one or more times. For example, it may be useful to repeat the process one or more times where the sample mixture has been fractionated (e.g. separated by chromatography or electrophoresis). By repeating the process on each sample, it is possible to analyze ~~all~~ the entire sample mixture. It is contemplated that in some embodiments, the whole process will be repeated one or more times and within each of these repeats, certain steps will also be repeated one or more times such as described above. In this way, the contents of sample mixture can be interrogated and determined to the fullest possible extent.

Please amend the paragraph at page 37, lines 15-21 as follows:

In some embodiments, methods of the invention can further comprise digesting each sample with at least one enzyme to partially, or fully, degrade components of the sample prior to performing the labeling of the analytes of the sample (Also see the above section entitled: "Sample Processing"). For example, the enzyme can be a protease (to degrade proteins and peptides) or a nuclease (to degrade nucleic acids). The enzymes may also be used together to thereby degrade sample components. The enzyme can be a proteolytic enzyme such as trypsin, papain, pepsin, ArgC, LysC, V8 protease, AspN, pronase, chymotrypsin or carboxypeptid[e]ase C.

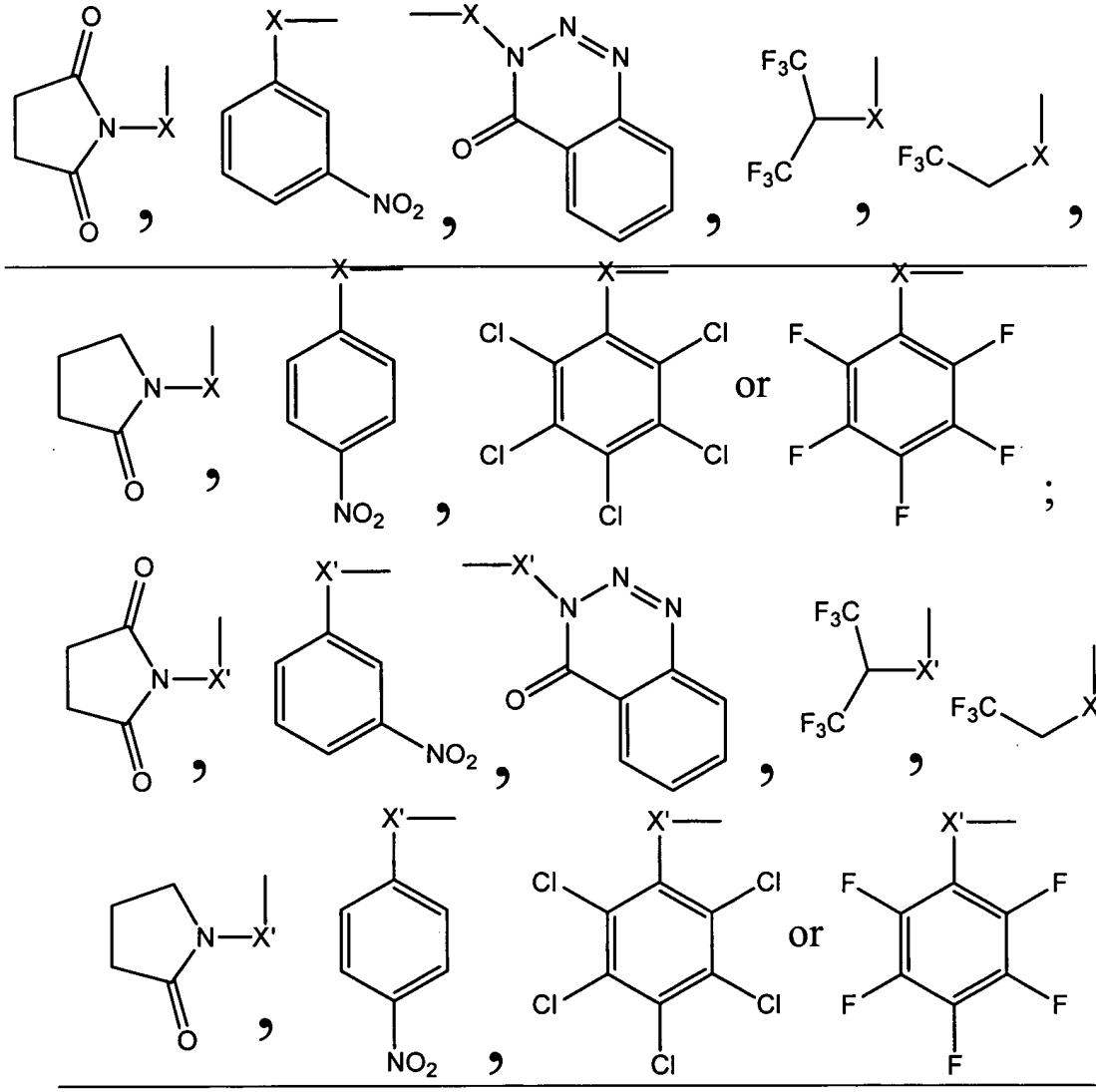
Please amend the paragraph at page 49, lines 3-6 as follows:

In some embodiments, the kit comprises a proteolytic enzyme. The proteolytic enzyme can be trypsin, papain, pepsin, ArgC, LysC, V8 protease, AspN, pronase, chymotrypsin or carboxypeptid[e]ase C. In some embodiments, the kit can comprise instructions for using the labeling reagents to differentially label the analytes of different samples.

Please amend the paragraph at page 50, lines 12-17 as follows:

In some embodiments, the labeling reagents can comprise a carbonyl or thiocarbonyl linker. Labeling reagents comprising a carbonyl or thiocarbonyl linker can be used in active ester form for the labeling of analytes. In an active ester, an alcohol

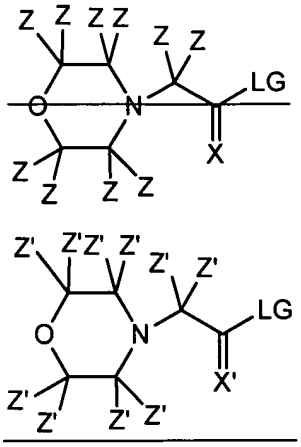
group forms a leaving group (LG). In some embodiments, the alcohol (LG) of the active ester can have the formula:



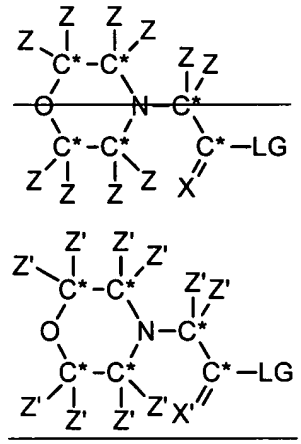
wherein X' is O or S. The active ester can be an N-hydroxysuccinimidyl ester.

Please amend the paragraphs at page 51, line 5 to page 53, line 14 as follows:

In some embodiments, the active ester compound can be an N-substituted morpholine acetic acid active ester compound of the formula:



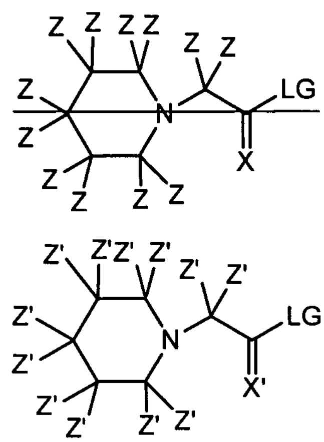
or a salt thereof, wherein; LG is the leaving group of an active ester; X' is O or S; each Z' is independently hydrogen, deuterium, fluorine, chlorine, bromine, iodine, an amino acid side chain or a straight chain or branched C1-C6 alkyl group that may optionally contain a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups each independently comprise linked hydrogen, deuterium or fluorine atoms. In some embodiments, Z' independently can be hydrogen, deuterium, fluorine, chlorine, bromine or iodine. In some embodiments, Z' independently can be hydrogen, methyl or methoxy. In some embodiments, X' is ^{16}O or ^{18}O. The nitrogen atom of the morpholine ring can be ^{14}N or ^{15}N. In some embodiments, the active ester is a compound comprising the formula:



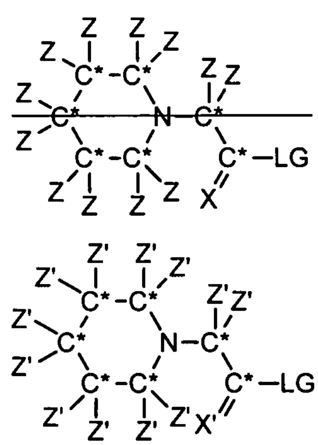
wherein each C* is independently ^{12}C or ^{13}C; LG is the leaving group of an active ester; X' is O or S; and each Z' is independently hydrogen, deuterium, fluorine, chlorine, bromine, iodine, an amino acid side chain or a straight chain or branched C1-C6 alkyl group that may optionally contain a substituted or unsubstituted aryl group wherein the carbon

atoms of the alkyl and aryl groups each independently comprise linked hydrogen, deuterium or fluorine atoms.

In some embodiments, the active ester compound can be an N-substituted piperidine acetic acid active ester compound of the formula:

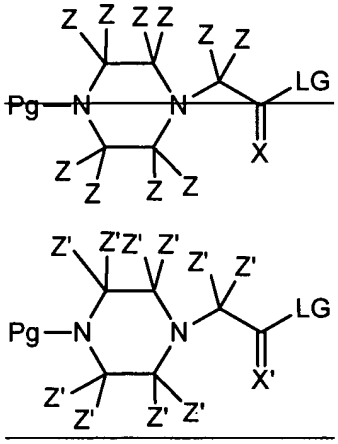


or a salt thereof, wherein; LG is the leaving group of an active ester; X' is O or S; each Z' is independently hydrogen, deuterium, fluorine, chlorine, bromine, iodine, an amino acid side chain or a straight chain or branched C1-C6 alkyl group that may optionally contain a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups each independently comprise linked hydrogen, deuterium or fluorine atoms. In some embodiments, Z' independently can be hydrogen, deuterium, fluorine, chlorine, bromine or iodine. In some embodiments, Z' independently can be hydrogen, methyl or methoxy. In some embodiments X' is ^{16}O or ^{18}O. The nitrogen atom of the piperidine ring can be ^{14}N or ^{15}N. In some embodiments, the active ester is a compound comprising the formula:

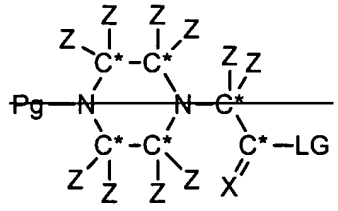


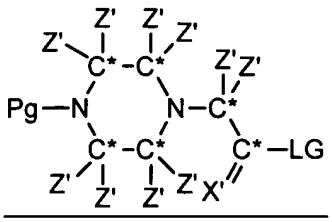
wherein each C* is independently ^{12}C or ^{13}C; LG is the leaving group of an active ester; X' is O or S; and each Z' is independently hydrogen, deuterium, fluorine, chlorine, bromine, iodine, an amino acid side chain or a straight chain or branched C1-C6 alkyl group that may optionally contain a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups each independently comprise linked hydrogen, deuterium or fluorine atoms.

In some embodiments, the active ester compound can be an N-substituted piperidine acetic acid active ester compound of the formula:



or a salt thereof, wherein; LG is the leaving group of an active ester; X' is O or S; Pg is an amine protecting group; and each Z' is independently hydrogen, deuterium, fluorine, chlorine, bromine, iodine, an amino acid side chain or a straight chain or branched C1-C6 alkyl group that may optionally contain a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups each independently comprise linked hydrogen, deuterium or fluorine atoms. In some embodiments, Z' independently can be hydrogen, deuterium, fluorine, chlorine, bromine or iodine. In some embodiments, Z' independently can be hydrogen, methyl or methoxy. In some embodiments X' is ^{16}O or ^{18}O. In some embodiments, each nitrogen atom of the piperazine ring is ^{14}N or ^{15}N. In some embodiments, the active ester is a compound comprising the formula:





wherein each C* is independently ^{12}C or ^{13}C; LG is the leaving group of an active ester; X'_ is O or S; Pg is an amine protecting group and each Z'_ is independently hydrogen, deuterium, fluorine, chlorine, bromine, iodine, an amino acid side chain or a straight chain or branched C1-C6 alkyl group that may optionally contain a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups each independently comprise linked hydrogen, deuterium or fluorine atoms.